Extract of *Urgenia Grandiflora* Inhibited Breast Cancer Cell (MCF-7) Proliferation and Tumorgenesity

Ibrahim B. E. El Bashir [A,B], Loiy Elsir Ahmed Hassan [C], Amin M. S. Abdul Majid [D], Sakina Yagi [A*]

Abstract

Objective: To investigate the cytotoxicity of *Urgenia grandiflora* bulbs towards three cancer cell lines; human colorectal carcinoma cell line (HCT 116), human hormone sensitive and invasive breast cancer cell line (MCF-7) and human hormone resistant breast cancer cell line (MDA-MB-231), in addition to endothelial normal EA.hy926 cell line. According to selective antiproliferative effect against MCF-7 breast cancer, *U. grandiflora* extract was subjected to apoptosis and antitumorgenesity studies on MCF-7 cell line. **Methods:** Maceration with chloroform: methanol (1:1, v/v) was performed to obtain crude extract. Cytotoxicity was established by colorimetric measurement of cell viability. The effect of the extract on mitochondrial membrane potential, chromatin condensation and nuclear morphology of MCF-7 cells were evaluated using Hoechst 33342 stain. The antitumorgenesity was also determined by evaluation of the ability of extract to suppress the reproductive potential of cell division and colonization after treatment (colongenicity). The effect of extract on migration of tumor cells from their primary growth site to distant locations was evaluated by the wound healing assay. Effect of extract on invasion of matrigel by MCF-7 cells was evaluated using standard methods. **Results:** *U. grandiflora* extract showed tumor-specific antiprolifera-tive activity against MCF-7 cells. the extract was also found to exert some toxicity towards the normal cell line EA.hy926 and this could be more likely attributed to its richness in cardiac glycosides. The extract demonstrated programmed cell death features, as it induced cell condensation, membrane flubbing and DNA fragmentation, also it disrupted mitochondria integrity in treated cells. Moreover the extract profoundly inhibited tumorgenesity of MCF-7 via inhibition of cell migration colony formation and cell invasion. **Conclusion:** *U. grandiflora* could be a new source of chemotherapeutic for breast cancer.

Key Words: *Urgenia grandiflora,* anticancer, apoptosis, antitumorgenesis *in vitro*

Significance | *Ureginea grandiflora* has a potential chemotherapeutic effect against breast cancer.

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Introduction

Cancer characterized by aggressive uncontrolled growth of malignant cells, which acquired the ability to invade and spread to distance organs (Nath et.al 2013). The International Agency for Research on Cancer characterized by aggressive uncontrolled growth of malignant cells, which acquired the ability to invade and spread to distance organs (Nath et.al 2013). The International Agency for Research on Cancer (IARC) estimated that about 14.9 million worldwide are cancer patients in 2013, of these 7.7 million were men and 6.9 million

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were women and by 2035 this number is expected to reach 24 million (Bray et.al 2013). The morbidity of cancer dramatically increased in recent years, every month the Atomic hospital receive 1000 cases, malignancies ranked as the major cause of death in the country (Elebead et al, 2012, Elamin et. Al, 2015). The most frequently mentioned cancers in Sudan were breast 24.8%, leukemia 17%, colon 15%, prostate 14.6%, oral 14.3%, lung13.9%, cervix 10.6%, stomach 9.6% and other scattered cancers (Ahmed et al, 2014).

Conventional treatment approaches have many limitations and require novel therapeutic agents to combat resistance, side effects, and carcinogenesis itself (Foo and Michor, 2014). Natural compounds from plant source can prevent, suppress, or reverse the progression of cancer, which represent excellent option for cancer prevention and treatment. In fact, medicinal herbs have a long history of use in the treatment of cancer. In review article, Hartwell listed more than 3000 plant species that have reportedly been used in the treatment of cancer (Hartwell, 1982). The search for cancer therapy from plants sources stated in 1950s with the discovery vinca alkaloids (vinblastine & vincristine) and the isolation of the cytotoxic podophyllotoxins (Cragg and, Newmann, 2005; Newman and Cragg, 2007). Saeed et al (2015) evaluated the cytotoxicity of 65 extracts from 35 plants, which are most frequently used in Sudanese traditional medicine for diverse indication including cancer-related symptoms, and they found that *Lawsonia inermis, Trigonella foenum-graecum* and *Ambrosia maritma* were the most active crude extracts.

Urginea grandiflora (Family: Hyacinthaceae) is perennial, herbaceous and bulbous plant, distributed in the Red Sea Hills in Eastern Sudan (Andrews FW, 1956). The latex of *U. grandiflora* obtained from the bulb of the plant used for wound hygiene and wound healing (Sultan et al., 2010). Few studies done on this species, however recently the *Urginea maritima* (same genus) studied for its cytotoxicity effects among sixty-one Egyptian medicinal plants (El-Seedi et al., 2013). The profound results inspired us to investigate *U. grandilora*. In the present study *U. grandiflora* bulb was screened for its antiproliferative potentials against three cancer cell lines namely; human colorectal carcinoma cell line (HCT 116), human hormone sensitive and invasive breast cancer cell line (MCF 7) and human hormone resistant breast cancer cell line (MDA-MB-231), in addition to normal endothelial EA.hy296 cell line. Selective cytotoxicity was found against MCF-7 cell line and thus the effect of *U. grandiflora* on apoptosis and antitumorgenesity of this cells line were conducted.

2. Materials and Methods

2.1 Plant materials

Plant material was collected from eastern Sudan, Erkowit region, in January/2015. Botanical identification and authentication were performed and voucher specimens (No. 2014/UG) have been deposited in Botany Department Herbarium, Faculty of Science, University of Khartoum, Sudan.

2.2 Extract preparation

The bulbs of *Ureginea grandiflora* were cut into small slices, air dried and grinded to powder. 40 g of plant material was macerated in ratio of organic solvents (chloroform: methanol) (1:1, v/v) (200 mL), at room temperature for 72 h. The filtrate was collected and concentrated at 45°C under vacuum by rotary evaporator (Buchi, USA) and further dried overnight at 45°C to obtain (6.952g). Stock solution of the extract was prepared at 10 mg/mL in 100% dimethyl sulfoxide (DMSO). The stock solution as well as DMSO (vehicle) was diluted with cell culture medium, so the highest DMSO concentration exposed to the cells was 0.1 % v/v.

2.3 Preliminary phytochemical screening

The extract was subjected to phytochemical analysis for the detection of terpenoids, alkaloids, saponins (Harborne Jb, 1984), tannins, flavonoids (Sofowora A, 1993), cardiac glycoside and anthraquinones (Trease and Evans, 1989) using standard phytochemical methods.

2.4 Cell viability assay

2.4.1 Cell lines and cell culture

Human Endothelial Cell line (EA.hy 926) purchased from ScienCell, USA. Human colorectal carcinoma cell line (HCT-116), human hormone sensitive and invasive breast cancer line (MCF-7) and human hormone resistant breast cancer cell line (MDA-MB 231) were purchase from ACCT, USA. All cells maintained in an incubator (Binder, Germany) with temperature 37°C, 5% CO_2 and humidity. EA.hy 926, MDA-MB 231 and MCF-7 were propagated in DMEM (Dulbecco's Modified Eagle Medium, Sigma, Germany) supplemented with 10% FBS (Foetal Bovine Serum, Sigma, Germany) and 1% PS (Penicillin/ Streptomycin, Sigma, Germany). HCT-116 was propagated in RPMI-1640 (Sigma, Germany) supplemented with 10% FBS, and 1% PS. Cell culture work was done in sterile conditions using Class II biosafety cabinet (ESCO, USA).

2.4.2 Cell proliferation assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromid) cytotoxicity assay was carried out according to the method described by (Mosmann, 1983) with minor modifications. Cells were seeded at $1.5x10^4$ cells in each well of 96-well plate in 100µL of fresh culture medium and were allowed to attach for overnight. The stock solutions of extracts were diluted in cell culture medium to obtain 100µg/mL and 100µL was added to each well. After 48 h of treatment the medium was aspirated and the cells were exposed to MTT solution prepared at 5mg /mL in sterile PBS added to each well at 10% v/v in respective medium and was incubated at 37°C in 5% CO_2 for 3 h. The water insoluble formazan salts was solubilized with 200µL DSMO/well. Absorbance was measured by Magellan – Microplate Reader Software TECAN Group Ltd., Switzerland) at primary wave length of 570 nm and reference wavelength of 620 nm. Each plate contained the samples, negative control and blank. DMSO at less than 1% v/v was used as a negative control. Similarly, tamoxifen with serial concentrations is used as standard reference drug.

2. Determination of changes in mitochondrial membrane potential

The changes in mitochondrial membrane potential was detected by Rhodamine 123 staining (Johnson et al., 1980; O'Connor et al., 1988). Briefly, MCF-7 cells (0.5 × 10^6 cells/mL) were cultured in 24-well plates. After cell attachment, the cells were exposed to new medium with extract (10 and 20µg/mL) or with vehicle (0.1% DMSO). The cells were washed twice with PBS, fixed with 4% paraformaldehyde for 30 minutes, washed with PBS, and stained for 30 minutes with Hoechst 33258 stain at 10µg/mL and Rhodamine 123 at 5µg/mL simultaneously. The cells were washed twice and observed under the EVOS f1 fluorescent digital microscope (Advanced Microscopy Group, USA). The loss of mitochondrial membrane potential was indicated by the loss of fluorescent intensity in the cells. The images were acquired after 6, and 12 hours. The apoptotic cells were counted in four randomLy selected fields per well. The apoptotic index was calculated as the percentage of apoptotic cells compared to the total number of cells.

2.6 Determination of nuclear condensation by Hoechst 33342 stain

The effect of *U. grandiflora* extract on nuclear fragmentation of MCF-7 was detected by fluorescence microscopy using Hoechst 33258 stain (Oberhammer et al., 1994: Zhang and Xu 2000). Cells were treated with extract (10 and 20µg/mL) and analyzed separately at two different time intervals (12 and 24 hours). 5-furoracel (10µg/mL) used as the positive and 0.1% DMSO as a negative control. The cells were fixed in 4% paraformaldehyde for 30 minutes before staining with Hoechst 33342 stain and incubated for 30 minutes. Nuclear condensation and fragmentation were examined under a fluorescence microscope. Cells with intensely colored, crescent shape, condensed, or fragmented nuclei were considered to be apoptotic. The number of cells with apoptotic morphology was counted in ten randomly selected fields per well. The cells were photographed at 20 × magnifications using an EVOS fluorescent digital microscope (Advanced Microscopy Group, Bothell, USA). The apoptotic index was calculated as the ratio of apoptotic cells to the total number of cells.

2.7 Clonogenicity assay

Effect on clonogenicity of MCF-7 cells evaluated by the colony formation assay as previously described by Franken et al (2006). Briefly, cells were harvested and resuspended in fresh DMEM culture medium at density 500 cell/ mL then cells were seeded in 6-well plate at 2 mL/well and incubated at 37°C and humidified 5% CO_2 for 24 hr. The old media was removed and cells were treated with different concentrations of the extract 2.5,5, 10 and 20 µg/mL in fresh medium. None treated cells were served as negative control and Tamoxifen as positive control. After 48 hr, the medium containing the treatment sample was removed and the cells were washed twice with PBS and a fresh medium was added. Then the cells were incubated for 5 days to allow colonies to form. At the end of incubation, the colonies were fixed in 4% paraformaldehyde for 30 min and stained with 0.25% crystal violet, the colonies were washed to remove the free excess stain and the number of colonies of more than 50 cells was counted using AMG EVOS florescence inverted microscope (4x10 magnification).

The plating efficiency (PE) of untreated cells was determined as follow:

PE = (the number of colonies formed / the number of cells seeded) x 100%.

The survival fraction (SF) of treated cells was calculated using the formula:

SF= ((number of colonies formed after treatment)/ (number of seeded cells x PE)) X 100%

2.8 Migration assay

The assay was carried out as described previously Liang et al. (2007). In brief, MCF-7 were seeded in 6 well plates till the formation of a confluent monolayer after which a wound was created using 200 µL micropipette tip. The detached cells were removed by washing with PBS and the plates were treated with *U. grandiflora* extract (10µg/ml). The wounds were photo-graphed after 12 and 18 h, and the width of the cell-free wounds was measured using an inverted light microscope supplied with Leica Quin computerized imaging system. Five fields per well were photographed and minimum of 15 readings per field were taken. The results are presented as mean percentage of migration inhibition compared to control.

2.9 Invasion assay

The assay carried out by a miner modification of the Boyden chamber assay using matrigel (Shaw et al., 2006; Baharetha, 2012). Matrigel was used as an artificial basement membrane matrix, this assay is more rigoroustest and it mimics the behaviour of transmigration of cells in vivo. Briefly, 50µL of (1:1 thawed matrigel (10 mg/mL in DMEM medium) was spread into 96-well plate and allowed to solidify by incubation at 37°C in 5% CO2 for 45min. Then MCF-7 cell line were suspended in DMEM medium and immediately seeded at 5×10^3 cells/well (150 µL/ well). Some of the cells were seeded in DMEM and DMSO only (1%) and used as a negative control, other wells were seeded with DMEM containing the extract (5,10 and 20 µg/mL), then re-incubated in the humidified incubator at 37°C and 5% CO2 for further 24 hours. Subsequently, the cell culture medium was aspirated carefully to remove the floating and dead cells. After a single wash with PBS the wells were captured using an AMG EVOS florescence inverted microscope (4xmagnification). Quantification of invasion was assessed by counting the number of the invaded cells in the treated wells with comparison to that of the negative control. The number of invading cells was determined, and results are presented as a percentage inhibition relative to the untreated cells. The calculations done as below:

% of inhibition of invasion = (1-(No. of invaded cells(T) /No. of invaded cells (–ve control))) × 100

Where, T: Treated wells with extract.

2.10 Statistical analysis

Each assay was repeated thrice independently with six replicate each. The results were presented as the mean ± standard error of mean (SEM). Fifty percent inhibitory concentration (IC_{50}) values were calculated from concentration-dependent curves using regression analysis in Microsoft Excel 2013. The statistical significance of difference was evaluated by analysis of variance (ANOVA), followed by Tukey's post hoc test. A P-value of less than 0.05 was considered significant.

3. Results

3.1 Phytochemistry

Phytochemical screening showed that the bulb of *U. grandiflora* is rich in cardiac glycosides. Flavonoids, tannin, alkaloids, saponins, terpenes and sterols were also detected while anthraquinones were absent.

3.2 Effect on cell viability

Extract of *U. grandiflora* bulb was tested against the three cancerous cells lines MCF7, HCT-116 and MDA-MB 231 and normal human endothelial cell line, EA.hy926 and results are presented in Table 1. The extract displayed obvious evidence of cytotoxicity on concentration dependent manner against MCF7 and EA.hy 926 cells with IC_{50} values 10.91 and 19.86 µg/mL respectively. The extract was less toxic to the other two cells lines (HCT-116 and MDA-MB 231) where the IC_{50} value was > 50 µg/mL. Photographs showing the effect of *U. grandiflora* extract on MCF-7 cells were also taken and the cytotoxic effect of the extract appeared in acute reduction in the number of cells due to inhibition the cell viability. The untreated cells displayed a compact monolayer of growing cancer cells (Fig.1).

3.3 Effect on mitochondrial membrane potential and nuclear morphology

The effect of *U. grandiflora* bulb extract at concentrations 5, 10 and 20 µg/mL for 6, 12 and 24 hours on mitochondrial membrane potential of MCF-7 cells was investigated by staining the nucleus with rhodamine 123 stain. Treatment of MCF7 cells with *U. grandiflora* extract caused, in a dose dependent manner, loss of mitochondrial membrane potential (Fig. 2-a) appearance of nuclear shrinkage, chromatin condensation and nuclear fragmentation indicating signs of early and late apoptosis (Fig. 2-b). At higher concentrations, some cells also revealed the characteristic crescent-shaped nuclei, which is a typical apoptotic nuclear morphology. Apoptotic index of untreated cells was 7.47 ± 0.48, while significant (P = 0.0001) increase of apoptopic index in a concentration dependent manner was observed (Fig. 2-c).

3.4 Inhibition of clonogenicity of MCF-7 cells

The ability of *U. grandiflora* bulb extract, at 2.5, 5, 10 and 20 µg/mL, to suppress the reproductive potential of cell division and colonization after treatment for 48h was also investigated. Results are presented in Fig. 3. The clonogenicity study on MCF-7 cells indicated that *U. grandiflora* extract was cytotoxic at all concentrations used, as evidenced by the decrease in the survival fraction (SF). The plating efficiency (PE) of untreated cells was found to be 14.4 ± 0.91% and the SF of the treated cells was 1 ± 0.57 % at 2.5 µg/mL and 0% at 5, 10 and 20 µg/mL. The result was comparable with the standard reference, tamoxifen (10 µg/mL), which inhibited colony formation completely (SF = 0 %.).

3.5 Inhibition of cell migration

Migration of tumor cells from their primary growth site to distant locations is a crucial step in metastatic tumor growth. The effect of *U. grandiflora* bulb extract on cell migration was evaluated by the wound healing assay. Due to the successful migration of endothelial cells in the untreated group, the wound is almost closed after 15 h, whereas in the *U. grandiflora* extract treated group, the wound remained open even after 15 h of incubation. The results are presented as average percentage of wound closure which was 67 ± 0.12% and 82 ± 0.68 % after 12 and 15 hours respectively in the untreated cells. The wound closure in treated cells with *U. grandiflora* extract at concentrations 10 µg/mL was reduced significantly (P <0.001) to 8 ± 1.68% and 24 ± 1.22% after 12 and 15 hours respectively while higher extract concentration (20 µg/mL) reduced it significantly (P <0.001) to 4 ± 1.98 % and 6 ± 0.31 after 12 and 15 hours respectively (Fig 4-a and b).

3.6 Inhibition of cell invasion

The ability of *U. grandiflora* bulb extract to inhibit MCF7 cells to invade the surrounding tissues was performed on Matrigel matrix. After 24h of treatment with *U. grandiflora* bulb extract the inhibition in cell invasion was 26.21 ± 5.54%, 50.96 ± 5.12 and 74.42 ± 5.89% at 5,10 and 20 µg/mL respectively (Fig. 5) suggesting the capacity in dose dependent manner of *U. grandiflora* extract to inhibit MCF7 cells to invade the surrounding tissues.

4. Discussion

Cancer treatment involves blocking specific molecular mechanisms in tumor proliferation by inducing cell death, hindering cell cycle progression or/and inhibiting tumor invasion and angiogenesis (Al Dhaheri et al., 2013). In the present study, *U. grandiflora* extract showed tumor-specific antiproliferative activity against MCF-7 cells. Analysis of mitochondrial membrane potential and nucleus morphology in MCF-7 cells revealed that *U. grandiflora* extract induced apoptosis that could be achieved by activating apoptosis triggering signals that cause loss of mitochondrial membrane potential (Fig. 2-a), cell shrinkage, chromatin condensation, and fragmentation (Fig. 2-b) [27]. Furthermore, *U. grandiflora* extract effectively suppressed, in a dose-dependent manner, MCF-7 cells colony formation (Fig. 3- a and b).

Tumor cells shed daily in blood circulation as part of their movement, and continuously extravasate and proliferate into the secondary sites (Eguchi, 2001). *U. grandiflora* extract inhibited significantly (P <0.001) cell migration toward the closure of the wound scratch after 12 and 15 h (Fig. 4- a and b) and showed also a capacity to restrict invasion of MCF-7 cells (Fig. 5) into the Matrigel basement indicating its ability to suppress the motility of the breast cancer MCF-7 cells and

Table 1. Cytotoxicity of *Urginea grandiflora* extract against different cell lines.

Cell lines	Concentration (µg /mL)						IC_{50} (µg/mL)
	3.125	6.25	12.5	25	50	100	
	Inhibition (%)						
MCF-7	40.49 ±3.84	43.14±2.44	55.35±4.38	58.2±1.42	59.72±2.11	62.62±0.22	10.91
HCT-116	12.57±5.23	13.83±1.49	14.19±3.68	18.66±3.18	22.25±2.04	22.38±8.61	> 50
MDA-MB 231	15.8±5.04	18.03±4.53	28.98±5.42	38.28±6.48	48.88±1.99	48.08±0.49	> 50
EA-hy 926	38.58±2.57	40.61±6.24	49.42±3.04	53.85±6.83	54.53±2.62	58.79±1.12	19.86

Extract, chloroform: methanol (1:1, v/v); Values are expressed as mean ± SD.

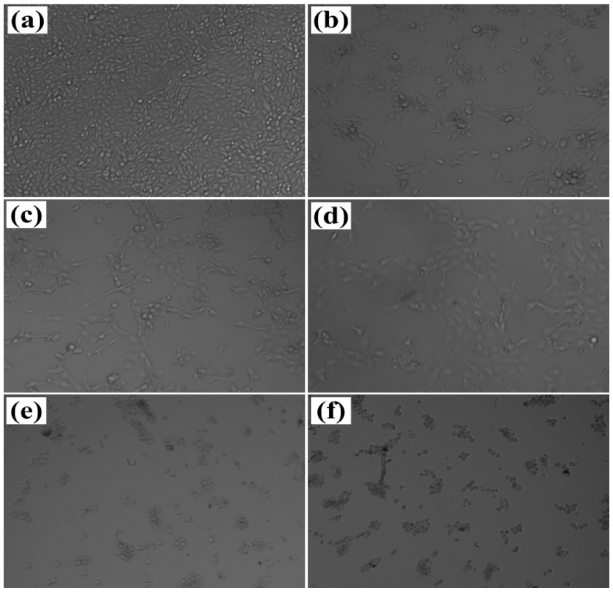

Figure 1. Cytotoxic effect of *Urginea grandiflora* bulb extract on the morphology of MCF7 cells. Morphological characteristics were observed after 24 h treatment with extract and the images were captured under an inverted phase-contrast microscope (200x magnification). (A) untreated cell; (B) Cells treated with 6.25 µg/mL of extract; (C) Cells treated with12.5 µg/mL of extract; (D) Cells treated with 25 µg/mL of extract; (E) Cells treated with 50 µg/mL of extract; (F) Cells treated with 100 µg/mL of extract. Values are expressed as mean ± SD. Plant extract (chloroform: methanol (1:1, v/v).

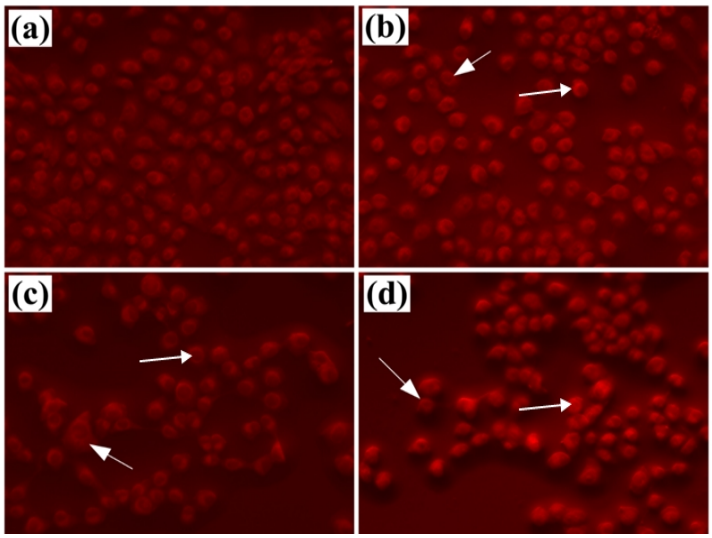

Figure 1-a. Effect of *Urginea grandiflora* bulb extract on mitochondrial membrane potential of MCF7Cells. (A) Untreated cells (control); (B) Cells treated with 5 µg/mL of extract; (C) Cells treated with10 µg/mL of extract; (D) Cells treated with 20 µg/mL of extract. Arrows indicates the apoptotic cells.

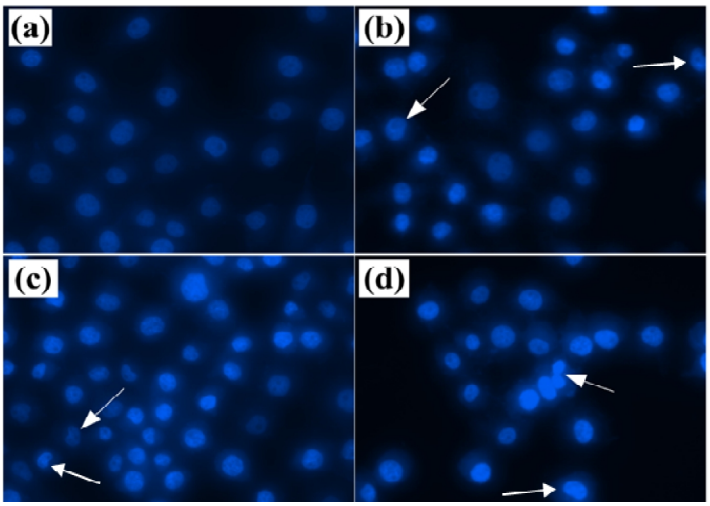

Figure 2-b. Effect of *Urginea grandiflora* bulb extract on chromatin condensation and nuclear morphology of MCF-7cells. (A) Untreated cells (control); (B) Cells treated with 5µg/mL of extract; (C) Cells treated with10 µg/mL of extract; (D) Cells treated with 20 µg/mL of extract. The extract treated cells exhibited obvious characteristic change of apoptosis. The arrows illustrate apoptotic bodies of the condensed, fragmented and crescent shaped nuclei and cells observed at 40× magnification under an inverted fluorescence microscope.

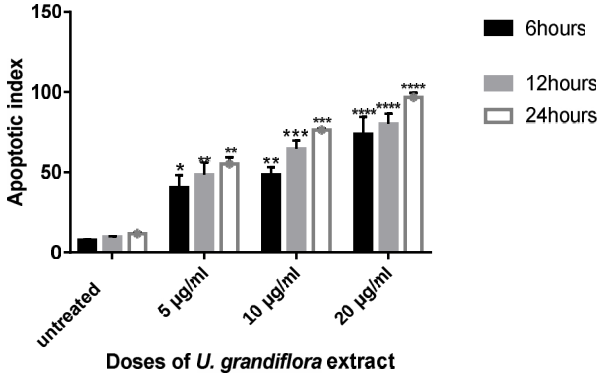

Figure2-c. Apoptotic index of MCF7 cells treated with *Urginea grandiflora* bulb extract. Plant extract (chloroform: methanol (1:1, v/v). (*p < 0.05, **p < 0.005 and ***p < 0.0005).

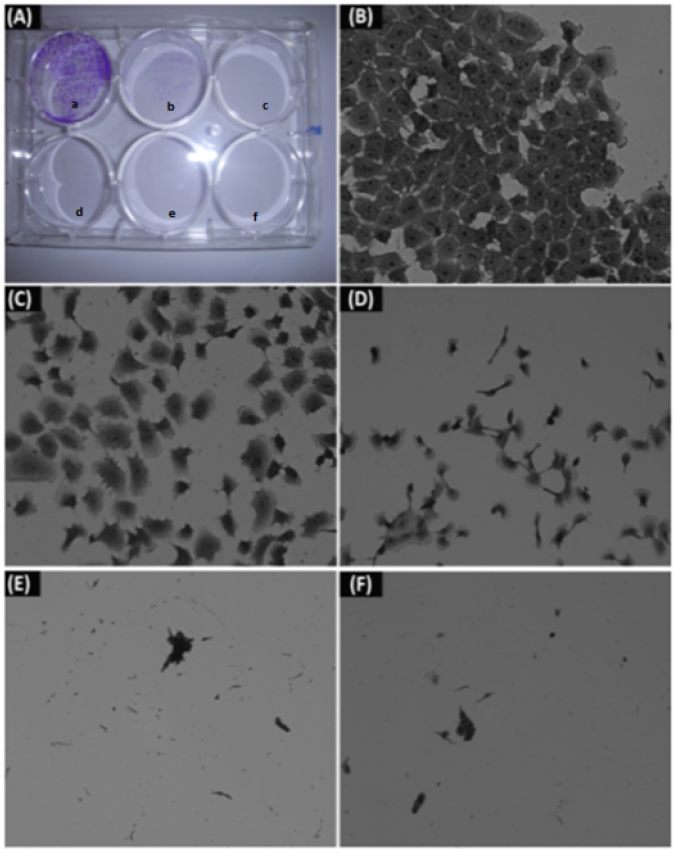

Figure 2-a. Photos indicating the inhibition in colongenicity of MCF7 cells caused by different doses *of Urginea grandiflora* bulb extract. (A) The effect of extract on the survival of MCF-7 colonies in colony formation assay. (A-a &B) Untreated cells; (A-b & C) Cells treated with 2.5 µg/mL; (A-c & D) Cells treated with 5 µg/mL; (A-d & E) Cells treated with 10 µg/mL; (A-e & F) Cells treated with 20 µg/mL; (A-f) Cells treated with tamoxifen. Cells (B-F) observed at 40× magnification under an inverted fluorescence microscope. Plant extracts (chloroform:methanol (1:1,v/v).

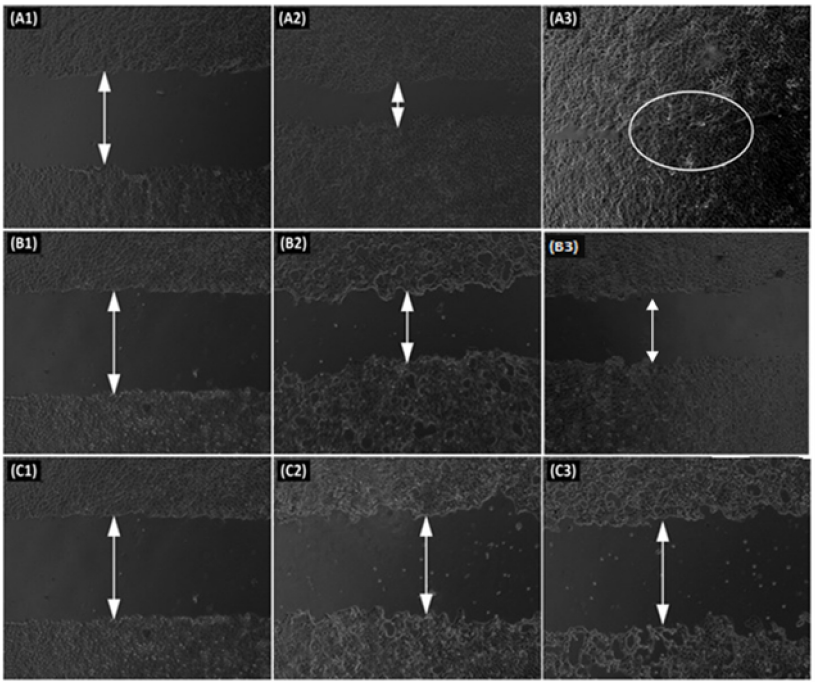

Figure 4-a. Photos shows the effect of *Urginea grandiflora* bulb extract on MCF7 cell migration ability. A (1-3) untreated Cell, B (1-3) Cells treated with 10 µg/mL of extract and C (1-3) Cells treated with 20 µg/mL of extract respectively at zero time (1), after12hrs (2)and after 15hrs (3).

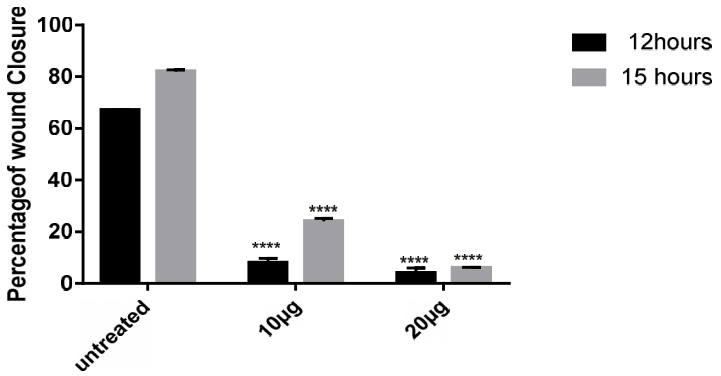

Figure 4-b. Effect of *Urginea grandiflora* bulb extract on MCF7 cell migration.
Plant extract (chloroform: methanol (1:1, v/v). (****$p < 0.05$)

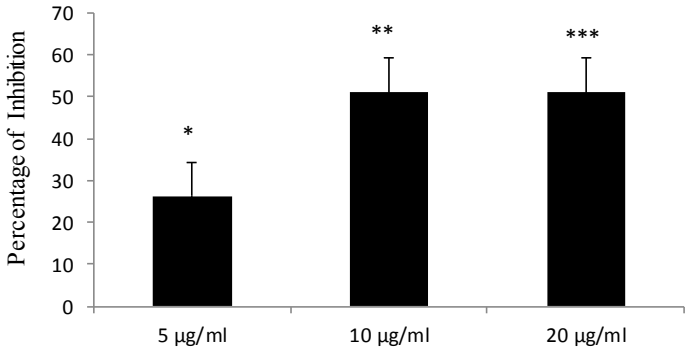

Figure 5. Effect of *Urginea grandiflora* bulb extract on MCF7 cell invasion.
Plant extract (chloroform: methanol (1:1, v/v). (*$p < 0.05$, **$p < 0.005$ and ***$p < 0.0005$).

thus suggesting its antimetastatic potential (Bockhorn,2007; Valster et al., 2005).

Thus, these results suggested that crude extract from bulb of *U. grandiflora* could be effective to inhibit cell proliferation and attenuate cancer invasiveness and motility. However, the extract was also found to exert some toxicity towards the normal cell line EA.hy926 and this could be more likely attributed to its richness in cardiac glycosides (Newman et al., 2005). Although, cardiac glycosides are known for their clinical uses to treat heart failure for many years (Prassas and Diamandis, 2008), their beneficial effect for the treatment of cancer is controversial. In a review article evaluating the cancer therapeutic potential of cardiac glycosides, Calderón-Montaño et al. (2014), reported some studies demonstrated that cardiac glycosides can inhibit the proliferation of both cancer cells and human nonmalignant at similar very low concentrations and consequently suggested their low potential for cancer therapy. Their toxicity against both cancerous and healthy human cells occur by their ability to inhibit the Na(þ)/K(þ)-pump and subsequent blocking of protein synthesis (Perne A et al., 2009). On the other hand, Babula P et al. (2013) and De S et al. (2016) highlighted in a review article the therapeutic role of cardiac glycosides in cancer treatment. Several alternative mechanisms compatible with their therapeutic use have been suggested particularly in terms of their pharmacodynamic interactions with cytotoxic drugs used in the clinic (Felth et al., 2009). Cardiac glycosides are known to influence the immune response at multiple levels (Kepp et al. 2012). They have antiproliferative activities via their regulation of the cell cycle and were found to selectively inhibited the proliferation of human tumor cells in mouse xenograft models through their effect on the complex mechanisms of cellular signal transduction (Newman et al., 2008; Balunas and Kinghorn 2005). They play an important role in angiogenesis by inhibiting the release of fibroblast growth factor-2 (FGF-2) (potent angiogenesis promoting substance) (De S et al. 2016).

No detailed phytochemical and cytotoxic studies were reported previously for *U. grandiflora,* however, Proscillaridin A, bufadienolide was isolated from *U. maritima* (El-Seedi et al., 2013) and was shown to inhibit the DNA topoisomerases I and II and increase the intracellular Ca^{2+} concentration Winnicka et al. (2010).

5. Conclusion

In conclusion, the present study indicated that *U. grandiflora* bulb crude extract inhibited significantly the growth, proliferation and invasion of human breast cancer (MCF-7) cells and induced apoptosis in a time-and-dose dependent manner. To the best of our knowledge this is the first detailed study that address the antitumor property of *U. grandiflora*. It is well known that; plant extracts comprise mixtures of complex metabolite which in turn exert their action on different levels and through several mechanisms (Winnicka et al., 2010). Thus, isolation and determination of bioactive molecule(s) responsible for inhibitory potential against breast cancer cells from bulb crude extract of *U. grandiflora* and their mode of action are warranted to evaluate their potential for cancer therapy.

Author Contributions

I.B.E.B. run the in vitro assay, L.E.A.H. assisted with experimental protocol and drafted the paper, A.M.S. designed the study, S.A.Y. drafted and revised the article.

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Competing financial interests

The author(s) declare no competing financial interests.

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